Body fat, energy balance and estradiol levels: a study based on hormonal proles from complete menstrual cycles

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Title: Body fat accumulation, energy balance and estradiol levels: a study based on hormonal profiles from entire menstrual cycles

Running title: Body fat, energy balance and estradiol levels in women

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Abstract

BACKGROUND

It is hypothesized that female fecundity is regulated by nutritional status. Although widely cited, this hypothesis is not strongly supported by empirical data from non-obese, healthy women of reproductive age.

METHODS

141 healthy, reproductive aged women from Southern Poland collected everyday morning saliva samples for one menstrual cycle. Levels of 17-β-estradiol were analyzed by radioimmunoassay. Measurements of body fat and anthropometrics were taken randomly with respect to phase of the menstrual cycle. Energy balance was specified basing on changes in percentage of body fat from the beginning to the end of the observation.

RESULTS

In women of very low to average body fat the 10% increase in fatness was associated with 5 to 7 pmol/l increase in estradiol levels. Women with high body fat had significantly lower levels of E2 comparing to women with low and average body fat.

In women with positive energy balance linear association between fatness and E2 was even stronger. Significant differences between body fat groups were also noted in estradiol profiles during menstrual cycle. In contrast, no such relationship was found in women with negative energy balance.

CONCLUSIONS

In healthy women body fat was positively associated with estradiol levels although this effect was restricted only to women with positive energy balance. These results point to the importance of negative energy balance as a factor affecting the relationship between fatness and estradiol levels.

Key words: 17-β-estradiol /body fat percentage/energy balance/menstrual cycle

Introduction

Even a relatively small decline in reproductive ovarian hormone levels during menstrual cycle has a marked effect on woman's fecundity (Ellison, 1991, Lipson & Ellison 1996). Estradiol, as the major and most active ovarian form of estrogen, is involved in ovarian follicle development, quality of the ovum and ovulation process. A small reduction in the levels of estradiol during follicular phase, without any significant change in the length of menstrual cycle, may lead to disturbed development of ovarian follicle, resulting in decreased probability of conception (Yoshimura and Wallach, 1987). Higher levels of follicular estradiol were observed in cycles resulting in conception comparing to cycles without conceptions in healthy subjects (Baird et al., 1997, 1999, Lipson and Ellison, 1996; Li et al., 2001, Lu et al., 1999; Venners et al 2006). In women undergoing in vitro fertilization higher levels of estradiol after the time of embryo transfer (Chen et al., 2003) or hCG administration (Blazar et al., 2004) were also strongly positively related to pregnancy success after the in vitro procedure.

Inter-individual variation in levels of steroid ovarian hormones can be attributed to several factors, including those related to energy availability and metabolism. While the influence of physical activity on ovarian hormone levels is relatively well recognized (Burrows and Bird, 2000; Chen and Brzyski, 1999; De Souza and Williams, 2004; Elias and Wilson, 1993; Jasienska, 2003; Jasienska and Ellison, 1998, 2004; Jasienska et al., 2006; Warren and Perlroth, 2001), the role of nutritional status is still controversial. In particular there is no agreement if the nutritional status can affect reproductive hormone levels independently from the influence of physical activity and energy balance, which were hypothesized to have predominant effect on reproductive physiology (Ellison, 2001, 2003, Jasienska, 2001, Jasienska and Ellison, 2004, Schneider, 2004, Wade and Jones, 2004, Wade et al., 1996).

The regulatory role of nutritional status on reproductive ability was first hypothesized by Frisch (Frisch, 1984) and the evidence on the relationship between nutritional status and ovarian hormone levels comes from studies on women with anorexia nervosa (Kirchengast and Huber, 2004; Miller et al., 2004, van Binsbergen et al., 1990, Warren, 1983). In these women, low body mass or low body mass index (BMI) is related to low levels of estradiol and inhibition of menstrual cycles. However, results of the studies on healthy, relatively well-nourished women are contradictory. Studies report no relationship (Dorgan et al., 1995; Ukkola et al., 2001, Ivandi et al., 1998; Panter-Brick et al., 1993), positive relationship (Barnett et al., 2001, 2002; Brunning et al., 1992; Furberg et al., 2005; Zanker and Swaine, 1998), or negative relationship (Howard et al., 1987, Potischman et al., 1996, Thomas et al., 1997a, b; Westhoff et al., 1996) between nutritional status and ovarian reproductive hormone levels, therefore it is not clear if biological variation in the reproductive hormone levels can be attributed to the influence of nutritional status.

However, several authors suggested that nutritional status cannot be considered as the factor regulating reproductive functioning independently from the effect of physical activity and energy balance (Ellison, 2001, 2003, Jasienska, 2001, Jasienska and Ellison, 2004, Wade and Jones, 2004). It is hypothesized that energy balance and physical activity have predominant role in reproductive function regulation. As energy balance affects nutritional status a relationship between nutritional status and estradiol can be in fact a by-product of the association between energy balance and reproductive hormone levels. In none of the cited studies the effect of physical activity and/or energy balance was accounted for thus it is impossible to determine if the observed changes in the levels of reproductive hormones are independently modified by the nutritional status.

Although recently several molecular models of the association between nutritional status and fecundity were proposed (Budak et al., 2006, Caprio et al., 2001, Rexford and Flier, 2000, Smith et al., 2002, Tatarani, 1997), conclusive results confirming this association are still lacking. Here, we report results of a study investigating an association between ovarian estradiol levels measured during entire menstrual cycle and nutritional status indicated by body fat percentage in 141 healthy, well-nourished, but not obese women from Southern Poland. To account for the confounding effect of energy balance separate analysis were conducted in sub-sample of 131 women for whom energy balance was estimated on the basis of changes in the body fat concentration. Our results suggest that in women with positive energy balance nutritional status has independent, regulatory effect on the levels of estradiol during menstrual cycles whereas in women with negative energy balance no such an effect can be observed.

Methods

Study Participants

The participants of the study were 141 women from urban and rural areas of Southern Poland. Women were recruited for the study by local media advertisement in the urban area, and through their parish in the rural area between June 2001 and June 2003. Selection of women for the study was based on the following criteria: age between 24 and 37 years, self-assessed regular menstrual cycles not shorter then 25 and not longer then 35 days, no fertility problems, no gynecological and endocrinological disorders, not taking hormonal oral contraceptives or other hormonal medications for the period of six months prior to the recruitment, and not being pregnant or lactating during the six months prior to the recruitment. The research protocol was approved by the Bioethical Committee of Jagiellonian University.

General questionnaire and physical activity assessment

General questionnaire collecting information about the place of birth, age, birth weight and birth length, age of menarche, education, marital status, reproductive history, use of hormonal medication, and tobacco consumption was distributed to the study participants.

Physical activity was assessed on the base of a pre-set daily log completed by women every day during their menstrual cycle. It collected data about hours of sleep, waking-up time, and time spent during the day on physical activities in five categories. Detailed description of the methods was published elsewhere (Jasienska et al 2006).

Anthropometric measurements and energy balance adjustment

Anthropometric measurements were taken from each woman twice, randomly with respects to the beginning and to the end of menstrual cycle. The maximal time elapsing from the first measurement to the beginning of the menstrual cycle and from the end of menstrual cycle to the second measurement was less then 65 days.

Measurements of height, weight, body fat percentage, breasts, underbreast, waist and hips circumferences were taken by the trained anthropologist. Body fat percentage was measured by bioimpedance using TANITA scale; model TBF 551 with measurement accuracy of 0.1%. Body mass was measured using the same Tanita scale, with the measurement accuracy of 0.1 kg. Body mass index (BMI) was calculated as ratio of height [m] to body mass [kg] squared. Waist and hips circumferences measurements were used to calculate waist-to-hip ratio (WHR). Breast and underbreast circumference measurements were used to calculate breast-to-underbreast ratio (BUR).

Energy balance was determined based on changes in the percentage of body fat between the first and the second measurement. A woman was classified as having positive energy balance when the difference in body fat percentage between the first and second measurement was equal or greater then -1%. When this difference was smaller then -1% a woman was classified as having negative energy balance

Salivary estradiol assay procedure and estradiol indices

Packages containing plastic vials and laboratory-tested chewing gum were distributed to women before the beginning of their menstrual cycle. During one entire menstrual cycle, every day in the morning after waking-up, women collected saliva samples to plastic tubes pretreated with sodium azide following published protocols (Lipson and Ellison, 1989). Saliva samples from 20 days (-5 to -24 reversed cycle days) were analyzed for E2 concentrations using RIA I-125-based kit (#39100, Diagnostic Systems Laboratories, Webster, Texas, USA) with published modifications to the manufacturer's protocol (Jasienska et al 2004). Average intra-assay variability was 9% and inter-assay variability varied from 23% for lower (15 pmol/l) to 13% for higher (50 pmol/l) values. The sensitivity of estradiol assay was 4 pmol/l. Before statistical analysis, days of cycles were aligned on the basis of midcycle drop day (day 0) identification, which provides a reasonable estimate of the day of ovulation (Lipson and Ellison, 1996). Values of E2 concentration from 18 consecutive days were used in the analysis, and the following estradiol indices were calculated: "mean E2" (mean of days from -9 to 8), "mid-cycle E2" (mean of days from -2 to 2), "day -1 E2 " (day -1 E2 value, day before the midcycle drop day), "day 0 E2" (day 0 E2 value, midcycle drop day), "mean follicular E2" (mean of days from -9 to -1) and "mean luteal E2" (mean of days from 0 to 8).

Statistical analysis

Women were divided into four groups based on the quartiles of the distribution of body fat percentage. The established designation of fatness quartiles was maintained in all analyses to allow comparisons between women groups (all women, positive energy balance women, negative energy balance women) and thus produced differences in sample sizes. Differences among very low, low, average and high body fat groups in basic characteristics i.e. age, size at birth, age at menarche, menstrual cycle length, body anthropometrics, body composition, physical activity and number of cigarettes smoked per day were tested by one-way factorial analysis of variance (ANOVA) followed by Duncan tests. Separate comparisons in basic characteristic were also made in body fat quartiles of women with negative and positive energy balance. The same procedure was applied to test the significance of the differences in E2 indices between body fat quartiles.

Additionally, simple regression models tested the effect of body fat on E2 levels, with each E2 index as the dependent variable and body fat as the independent predictor. Separate analyses were conducted for women with positive and negative energy balance.

Repeated measure analysis of variance was used to test the differences in E2 profiles among the body fat groups in positive energy balance women. Separate models for values of E2 levels during -9 to 8 days (whole cycle), -9 to -1 days (follicular phase) and 0 to 8 (luteal phase) as dependent variables and body fat group as the independent were tested. The contrast analyzes indicated statistical significance of differences among four body fat groups.

Results

BODY FAT AND ESTRADIOL

General characteristics

General descriptive statistics of all women categorized with respect to their fatness are presented in Table I. Women characterized by very low, low, average and high body fat did not differ significantly with respect to age, birth weight and birth height, age of first menstruation, number of cigarettes smoked per day and physical activity. They differed significantly with respect to usual length of menstrual cycle ($F_{3, 134}$ =2,73, $p<0.05$) and anthropometric traits. Women with average body fat had significantly shorter usual, self-reported length of menstrual cycles as compared to women with very low body fat (28.1 days for average body fat group vs 30.2 days for very low body fat group). As expected, women characterized by higher body fat content had significantly higher body mass, BMI, and higher waist-to-hip ratio (all within groups comparisons were statistically significant at $p<0.05$), but they did not differ significantly with respect to their breast-to-underbreast ratio.

Estradiol levels in body fat groups

Mean levels of 17-β-estradiol in subsequent body fat groups are presented in Table II. Comparing to women characterized by average body fat women with very low and high body fat percentage had significantly lower levels of estradiol during follicular phase (21.1 vs 17.0 and 15.9 pmol/l, $F_{3,124}$=3.22, p=0.025), midcycle (25.4 pmol/l vs 19.7 and 18.2 pmol/l, $F_{3, 126}$=4.03, p=0.009), and on day -1 (38.8 pmol/l vs 29.1 and 26.6

pmol/l, $F_{3, 123}$=4.60, p=0.004), day 0 (20.4 pmol/l vs 15.2 and 13.4 pmol/l, $F_{3, 122}$= 3.35, p=0.021). Similar differences were also observed in comparisons between women with low body fat and women with very low and high body fat.

Additional evidence for the relationship between nutritional status and estradiol levels came from simple regression analysis restricted to a group of non-overweight women (body fat <31%). In these women, we observed linear, positive association between the amount of accumulated body fat and mean E2 (R^2=0.085, p=0.003), follicular E2 (R^2=0.075, p=0.007), mid-cycle E2 (R^2=0.094, p=0.002) (Figure 1) and luteal E2 (R^2=0.076, p=0.006). Statistically significant relationship was also observed for E2 on day -1 (R^2=0.097, p=0.002) and on day 0 (R^2=0.085, p=0.004). The 10% increase in percentage of body fat in the range of 9.1 to 30.8% was associated with 7 pmol/l increase in mid-cycle E2 and 5 pmol/l increase in follicular phase E2, luteal phase E2 and mean E2 during the menstrual cycles.

BODY FAT ENERGY BALANCE AND ESTRADIOL LEVELS

General characteristics

To test the effect of energy balance on the relationship between body fat and estradiol separate analyzes were conducted in 131 women characterized by positive or negative energy balance. General characteristics of women from those groups are presented in Table III. Women with negative energy balance as compared to those with positive energy balance had significantly longer usual cycle length (30.0 days vs 28.8 days respectively) and lower body weight at birth (3.38 kg vs 3.07 kg). However, they did not differ significantly in any of the anthropometric or life style parameters.

In contrast significant differences in anthropometrics were observed within both negative and positive energy balance group between women characterized by very low, low, average and high body fat content. As expected, higher weight, body fat, body mass index and waist-to-hip ratio were observed in women with higher body fat content comparing to women with lower body fat content (all between group comparisons significant at $p<0.05$) in positive energy balance group. In negative energy balance the same trend was observed for weight, body mass index and body fat. Additionally, in positive energy balance group women with very low and high body fat content had significantly higher birth weight comparing to women with low and average body fat content (3.58 kg for very low body fat content group and 3.57 kg for high body fat content group vs 3.18 kg for low body fat content group and 3.21 kg for average body fat content group).

Estradiol levels in body fat and energy balance groups

Average values of consecutive E2 indices in body fat groups of women with positive and negative energy balance group are presented in Table IV. Anova analyzes of estradiol levels in these groups revealed significant differences in all estradiol indices between women differing with respect to body fat content in positive but not in negative energy balance group. Significantly lower levels of mean E2, mid-cycle E2, day -1 E2 and follicular phase E2 were observed in women with very low and high body fat content comparing to women with low and average body fat content. Additionally, levels of day 0 E2 and luteal phase E2 were significantly lower in women with very low and high body fat group in comparison with women with average body fat group.

Repeated measure analysis of variance revealed significant differences in estradiol profiles during menstrual cycle between body fat groups ($F_{3,66}=3.81$,$p<0.05$) in women with positive energy balance. Women with very low and high body fat had significantly lower levels of E2 during whole menstrual cycle comparing to women with average and high fat (Figure 2). Similar differences between body fat groups was also observed with respect to levels of E2 during follicular phase ($F_{3,66}=3.69$, $p<0.05$) and levels of E2 during luteal phase ($F_{3,66}=3.17$, $p<0.05$).

The relationship between body fat and E2 levels was further confirmed by regression analyzes in non-overweight women with positive but not in those with negative energy balance (Table V). In women with positive energy balance we observed linear association between amount of accumulated body fat and mean E2 ($R^2=0.13$, $p<0.01$), mid-cycle E2 ($R^2=0.15$, $p<0.01$), follicular E2 ($R^2=0.11$, $p<0.05$) and luteal E2 ($R^2=0.14$, $p<0.01$) (Figure 3). The same trend was also observed for estradiol on day -1 ($R^2=0.14$, $p<0.01$) and on day 0 ($R^2=0.14$, $p<0.01$). In the range of 9.1 to 30.8% of body fat, the 10% increase in body fat content was associated with about then 7 pmol/l increase in mean E2, luteal E2 and follicular E2. Also 10% increase in body fat content was associated with about 9 pmol/l increase in E2 on day 0, 10 pmol/l increase in midcycle E2 and almost 15 pmol/l increase in E2 on day -1.

Discussion

To our knowledge this is the first study which clearly demonstrates the relationship between the amount of fat accumulated in woman's body and the entire cycle estradiol profile in a relatively large sample of healthy, well-nourished but not obese women. This is also the first study, which demonstrates how this relationship can be modified by energy balance.

In healthy non-obese women, we found non-linear association between body fat percentage and levels of estradiol during menstrual cycle. Women with very low (below 22%) and high body fat (above 31%) had 25 to 35% lower levels of follicular and mid-cycle estradiol. Lower levels were also observed for days 0 and -1. Furthermore, when the analysis was restricted to women characterized by body fat percentage lower than 31%, we found a positive linear relationship between fat accumulation and estradiol levels. The 10% increase in accumulated body fat was associated with 5 to 7 pmol/l increase in estradiol levels.

Interesting differences in the association between body fat and estradiol levels were demonstrated between groups of women with positive and negative energy balance. Whereas in women with negative energy balance no relationship between body fat and estradiol levels was found, in women with positive energy balance the association was even stronger then in case of the whole sample of women. Significant differences between body fat groups were observed in all indices of estradiol. Women with very low and high body fat had 30 to 45% lower levels of E2 then women with low and average body fat. Additionally in non-overweight, positive energy balance women 10% increase in body fat was associated with 7 to even 15 pmol/l increase in estradiol levels.

Levels of estradiol during menstrual cycle and especially during its follicular phase are related to follicular diameter, oocyte quality and endometrium morphology and thickness (Cahill et al., 2000, Ohno and Fujimoto, 1998). Lower levels of E2 during the menstrual cycles and during ovulation co-occur very frequently with lower pregnancy rate both in healthy, naturally conceiving women (Li et al., 2001, Lipson and Ellison, 1996, Lu et al., 1999; Venners et al 2006) and women undergoing in vitro fertilization procedure (Blazar et al., 2004, Chen et al., 2003). It can be estimated that 25 to 35% difference in estradiol levels in very low and high body fat group observed in our study can be translated into 2 to 3 times lower probability of conception (Lipson and Ellison, 1996).

Results of our study clarify and extend the accumulated evidence on the association between nutritional status and levels of reproductive steroids. Several studies conducted on different groups of premenopausal women (diabetic, obese, dieting, very lean and normal weight) demonstrated contradictory results (Barnett et al., 2001, 2002; Brunning et al., 1992; Furberg et al., 2005; Zanker and Swaine, 1998, Howard et al., 1987, Potischman et al., 1996, Thomas et al., 1997a, b; Westhoff et al., 1996). This inconsistency partly results from methodological limitations, especially calculations of mean E2 levels based on no more than a few samples from each menstrual cycle. Due to substantial intra-cycle variation in E2 levels, such sampling is vastly insufficient and can lead to errors in estimating mean E2 levels for individual women (Jasienska & Jasienski in press). In most of the studies estradiol levels were analyzed from a single blood sample, whereas Williams et al. (2002), relying on repeated measurements of reproductive steroids in 34 women, showed that at least eight samples taken from a single subject are necessary to detect about 80% of biological variation in estradiol levels during a particular menstrual cycle. In our study, the number of samples taken from a single subject considerably exceeded this requirement.

Another limitation of other studies is using BMI as the indicator of nutritional status. BMI does not represent sufficient information about nutritional status and accumulated body fat (Kyle et al., 2003, Piers et al., 2000). Frequently, individuals classified as overweight on the basis on the BMI criteria are of normal adiposity, especially when characterized by high muscle mass (Hortobagyi et al., 1994, Nevill et al., 2005, Wit and Bush, 2005). Conversely, women classified as normal using BMI criteria frequently have increased adiposity (Frankenfield et al. 2001). In our study, this limitation was omitted by direct measurement of body fat percentage. Lack of relationship between estradiol and BMI noted in our study and positive relationship between E2 and percentage of body fat observed at the same time, provides further evidence that BMI may be a poor indicator of nutritional status.

Additionally to the careful measurements of estradiol levels and nutritional status assessment we were able to estimate the energy balance of women based on the changes in body fat during the observational period. This estimation allowed us to investigate the interactions between nutritional status, energy balance and the entire cycle estradiol levels, which to our knowledge were never reported before. Energy balance was shown to influence ovarian steroids profiles in several studies (Ellison et al., 1989, Lager and Ellison, 1990, Panter-Brick, 1993). Our results indicate that negative energy balance caused by increased physical activity and/or inadequate calories intake has confounding effect on the association between nutritional status and reproductive hormone levels. This fact can explain lack of the relationship between nutritional status and levels of reproductive hormones demonstrated in populations or groups of women characterized by high physical activity (Jasienska, 1998, Jasienska and Ellison, 2004, Lager and Ellison, 1990), women loosing weight due to voluntarily caloric restriction (Lager and Ellison, 1990) and women from hunter gatherer and horticulturalist groups in Africa and Nepal (Ellison et al, 1989, Panter-Brick, 1993) who experienced periods of restricted caloric intake and high physical activity. In contrast, association between body fat and reproductive hormones frequently was demonstrated in women from western population who generally have higher energy intake and lower physical activity, none of these studies however controlled energy balance (Barnett et al., 2001, 2002; Brunning et al., 1992; Furberg et al., 2005; Zanker and Swaine, 1998).

In our study, no linear association between E2 and body fat was found in a group of overweight women, but the levels of E2 in these women were significantly lower then in normal adiposity women. This finding corresponds with results of other studies concerned with levels of E2 in overweight and obese women. Drop in E2 surge was noted in studies by Grenman et al. (1986), Kopelman et al. (1980), Leenen et al. (1994) and recently by Tworoger et al. (2006). Several authors demonstrated that in women increased adiposity and obesity are related to high androgenic activity (Evans et al., 1983, Hauner et al., 1988, Norman and Clark 1998, Wabitsch et al., 1995). This may explain the inversion in the pattern of the association between body fat percentage and estradiol levels observed in our study. In accordance with our results, Wang et al. (2000) showed that in underweight (BMI <20) and overweight (BMI>25) women, the probability of pregnancy during assisted reproduction treatment was about 20% lower comparing to women of normal range of BMI. Our high body fat group corresponded closely to overweight category in study by Wang et al. (see body fat % to BMI recalculation in Gallagher et al, 2000). Additionally, recently Gesink Law et al. (2006) demonstrated significantly reduced fecundity in overweight and obese women of reproductive age. Since the estradiol levels are highly related both to endometrial thickness and ovum quality, our results suggest that lower fecundity, conception and pregnancy rates in underweight, overweight and obese women can be mediated by unfavorable estradiol environment.

Decreased levels of estradiol in overweight and obese women found in our study have significant clinical implications for breast cancer research. Recently, Baer et al. (2006) in the large cohort of premenopausal women demonstrated the significant relationship between the energy status during childhood and adolescence and the risk of breast cancer. In women from the most overweight group during the childhood and the adolescence the risk of premenopausal breast cancer was almost 50% lower then in women from the leanest group. The same pattern was also shown in study by Michels et al. (2006), in which BMI at the age of 18 was the strongest predictor of premenopausal breast cancer risk. Our results point to the possible hormonal mechanism of the observed relationship. Since the high estradiol level is regarded the major mediator of breast cancer and the body mass index highly correlates with the amount of accumulated body fat it can be hypothesized that the reduction in the premenopausal breast cancer risk is mediated through the reduction of the estradiol levels in overweight and obese women.

Our results confirm the hypothesis about the regulatory role of nutritional status on potential fertility in reproductive age women, but only in those with positive energy balance. In women of low and normal adiposity, we observed a positive association between the amount of accumulated body fat and levels of estradiol during menstrual cycle. Similar effect was demonstrated in several different studies (Barnett et al., 2001, 2002; Brunning et al., 1992; Zanker and Swaine, 1998), but in these studies nutrition status was assessed by BMI and they did not control for the effect of energy balance

In women of reproductive age, energetic resources are partitioned in order to maintain normal both physiological processes and reproduction. Increased energy requirements of reproductive processes can be partially supported by energetic reserves stored in women's bodies in the form of fat. This fat depot is formulated in favorable environmental conditions when energy intake is high, energy expenditure of physical activity is low and total energy balance is positive. Consequences of inadequate nutritional status of women during pregnancy are highly adverse both to the child and to the mother. Low body mass of a woman prior to and during pregnancy is associated with high risk of preterm labor, intrauterine growth retardation, low birth weight of an infant and maternal depletion syndrome (Ehrenberg et al., 2003; Jelliffe and Maddocks, 1964; Kramer, 2003; Winkvist et al., 1992). Short-term reproductive suppression in women with low energy reserves may function as an evolutionary adaptation protecting against these risks and improving chances of successful reproduction in the future (Ellison 1990, Ellison, 2001, Jasienska 2003).

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Table I

General characteristics of study participants. Mean and standard deviation (in parentheses) for four consecutive body fat groups compared by one-way ANOVA tests.

	All women	Very low body fat ≤22% N=36	Low body fat ≤26.5% N= 35	Average body fat ≤30.8% N= 35	High body fat >30.8 N=35
	N=141				
Age [years]	29.8 (3.34)	29.6 (3.07)	29.7 (3.75)	29.4 (3.25)	30.6 (3.25)
Age of menarche [years]	13.4 (1.31)	13.6 (1.37)	13.2 (1.26)	13.3 (1.35)	13.6 (1.28)
Usual cycle length [days]*	29.2 (3.13)	30.2 (3.82)	29.6 (3.52)	28.1 (2.16)	29.1 (2.46)
Birth weight [kg]*	3.27 (0.611)	3.34 (0.626)	3.22 (0.502)	3.24 (0.500)	3.28 (0.819)
Birth length [cm]	53.2 (4.38)	53.1 (3.74)	53.7 (2.90)	52.6 (4.37)	53.4 (6.37)
Height [cm]	163.1 (6.51)	161.7 (6.12)	162.1 (6.30)	164.2 (7.11)	164.3 (6.26)
Weight [kg]*	60.1 (8.37)	51.4 (3.67)	56.3 (3.10)	62.5 (4.40)	70.3 (6.15)
BMI [kg/m²]*	22.6 (2.84)	19.7 (1.25)	21.4 (1.47)	23.2 (1.65)	26.0 (1.92)
Body fat% *	26.4 (6.52)	17.9 (3.19)	24.4 (1.19)	28.8 (1.22)	34.6 (2.31)
BUR	1.16 (0.037)	1.15 (0.037)	1.15 (0.036)	1.16 (0.036)	1.17 (0.037)
WHR *	0.72 (0.043)	0.70 (0.029)	0.71 (0.037)	0.72 (0.041)	0.75 (0.051)
Physical activity [MET*hour/day]	5.8 (2.95)	4.7 (1.47)	6.1 (3.09)	5.9 (2.95)	6.4 (3.68)
Number of cigarettes	1.8 (4.44)	1.1 (3.65)	1.9 (4.30)	2.1 (5.20)	2.0 (4.59)

* differences between body fat groups at $p<0.05$

Table II

Estradiol indices in consecutive body fat groups. Mean and standard deviation (in parentheses) for four consecutive body fat groups compared by one-way ANOVA tests.

E2 [pmol/l]	All women N=130	Very low body fat N=34	Low body fat N=34	Average body fat N=31	High body fat N=31
mean E2	18.5 (8.99)	17.0 (8.45)	20.3 (9.70)	20.7 (9.32)	16.1 (7.87)
mid-cycle E2*	22.1 (11.43)	19.7 (10.54)	25.4 (11.77)	25.2 (12.51)	18.2 (9.20)
day -1 E2 *	33.3 (18.27)	29.1 (15.38)	39.2 (17.75)	38.8 (22.26)	26.6 (14.22)
day 0 E2 *	17.1 (11.36)	15.2 (10.33)	19.4 (11.74)	20.4 (13.16)	13.4 (8.70)
mean follicular E2 *	18.7 (8.97)	17.0 (8.08)	20.9 (10.27)	21.1 (9.71)	15.9 (6.56)
mean luteal E2	18.3 (9.84)	17.1 (9.60)	19.6 (10.01)	20.2 (9.39)	16.3 (10.26)

*differences between body fat groups significant at $p<0.05$

Table III

General characteristics in consecutive body fat quartiles of women with positive and negative energy balance. Mean and standard deviation (in parentheses) for four consecutive body fat groups compared by one-way ANOVA tests.

	All women N=131	Positive Energy Balance N=75	Negative Energy Balance N=56
Age [years]	29.9 (3.36)	30.1 (3.49)	29.6 (3.17)
Very low fat	29.4 (3.01)	29.9 (3.04)	28.9 (2.91)
Low fat	29.9 (3.77)	30.7 (3.83)	28.9 (3.58)
Average fat	29.1 (3.34)	28.7 (3.35)	29.8 (3.33)
High fat	30.9 (3.13)	31.3 (3.42)	30.5 (2.83)
Age of menarche [years]	13.4 (1.34)	13.3 (1.22)	13.5 (1.48)
Very low fat	13.7 (1.42)	13.5 (1.64)	13.9 (0.88)
Low fat	13.1 (1.29)	13.1 (1.20)	13.1 (1.46)
Average fat	13.2 (1.31)	13.3 (1.10)	13.0 (1.60)
High fat	13.7 (1.27)	13.5 (0.80)	13.9 (1.63)
Usual cycle length [days]**	29.3 (3.25)	28.8 (3.02)**	30.0 (3.45)**
Very low fat	30.3 (4.04)	29.3 (3.18)	32.3 (4.90)
Low fat	29.6 (3.64)	29.3 (3.97)	29.9 (3.23)
Average fat	28.3 (2.23)	27.8 (2.04)	29.0 (2.35)
High fat	29.0 (2.56)	28.6 (2.37)	29.4 (2.77)
Birth weight [kg]**	3.26 (0.627)	3.38 (0.500)**	3.07 (0.748)**
Very low fat	3.38 (0.641)	3.58 (0.547)	3.01 (0.702)
Low fat	3.25 (0.478)	3.18 (0.477)	3.34 (0.484)

Average fat	3.12 (0.519)	3.21 (0.493)	3.01 (0.554)
High fat	3.29 (0.847)	3.57 (0.349)	2.86 (1.177)
Birth length [cm]	53.3 (4.59)	53.8 (4.08)	52.5 (5.36)
Very low fat	53.3 (3.99)	53.7 (4.67)	52.5 (1.76)
Low fat	53.8 (2.66)	53.9 (2.94)	53.6 (2.32)
Average fat	52.9 (4.73)	52.9 (5.49)	53.0 (3.71)
High fat	53.0 (6.76)	54.7 (2.22)	50.5 (10.20)
Height [cm]	163.1 (6.53)	163.3 (6.71)	162.7 (6.33)
Very low fat	161.9 (6.11)	162.4 (6.62)	160.5 (5.04)
Low fat	162.6 (6.18)	162.3 (6.16)	162.9 (6.42)
Average fat	163.7 (7.29)	163.6 (7.07)	163.9 (7.84)
High fat	164.2 (6.53)	165.1 (7.21)	163.2 (5.80)
Weight [kg]*	60.0 (8.40)	60.0 (8.51)	60.1 (8.39)
Very low fat	51.5 (3.64)	51.4 (3.72)	51.1 (3.45)
Low fat	55.9 (3.03)	56.1 (3.31)	55.6 (2.70)
Average fat	62.3 (4.61)	62.6 (3.81)	61.9 (5.64)
High fat	70.1 (6.39)	71.1 (6.44)	69.2 (6.38)
BMI [kg/m²]*	22.4 (3.43)	22.5 (2.90)	22.3 (4.07)
Very low fat	19.7 (1.30)	19.5 (1.33)	19.9 (1.34)
Low fat	21.2 (1.34)	21.3 (1.35)	21.0 (1.36)
Average fat	22.6 (4.20)	23.5 (1.57)	21.5 (6.13)
High fat	26.0 (2.00)	26.1 (2.10)	26.0 (1.95)
Body fat% *	26.3 (6.49)	25.9 (6.57)	26.9 (6.29)
Very low fat	17.6 (3.04)	17.2 (2.84)	18.4 (3.40)

Low fat	24.1 (1.23)	24.2 (1.19)	24.0 (1.32)
Average fat	28.6 (1.31)	28.7 (1.19)	28.4 (1.48)
High fat	34.4 (2.30)	34.4 (2.38)	34.4 (2.30)
BUR	1.16 (0.037)	1.16 (0.034)	1.16 (0.039)
Very low fat	1.15 (0.039)	1.14 (0.027)	1.17 (0.052)
Low fat	1.16 (0.037)	1.15 (0.032)	1.16 (0.042)
Average fat	1.16 (0.035)	1.16 (0.038)	1.15 (0.029)
High fat	1.17 (0.036)	1.17 (0.038)	1.17 (0.035)
WHR *	0.72 (0.044)	0.72 (0.046)	0.71 (0.042)
Very low fat	0.70 (0.029)	0.70 (0.027)	0.71 (0.034)
Low fat	0.70 (0.035)	0.71 (0.028)	0.70 (0.044)
Average fat	0.72 (0.043)	0.73 (0.044)	0.71 (0.039)
High fat	0.75 (0.053)	0.76 (0.055)	0.73 (0.046)
Physical activity [MET*hour/day]*	6.0 (3.00)	6.4 (3.22)	5.4 (2.59)
Very low fat*	4.7 (1.47)	5.1 (1.37)	3.9 (1.28)
Low fat	5.9 (2.90)	6.6 (3.15)	5.0 (2.33)
Average fat	6.6 (3.10)	6.7 (3.25)	6.5 (2.97)
High fat	6.6 (3.71)	7.2 (4.42)	6.0 (2.76)
Number of cigarettes	1.3 (4.01)	1.2 (3.46)	1.6 (4.79)
Very low fat	0.7 (2.46)	1.2 (3.10)	0.0 (0.00)
Low fat	1.4 (3.98)	1.3 (2.68)	1.6 (5.43)
Average fat	1.3 (4.23)	0.6 (2.42)	2.2 (5.94)
High fat	2.0 (5.15)	1.9 (5.39)	2.2 (5.07)

* differences between body fat quartiles significant at $p<0.001$

** differences between positive and negative energy balance significant at $p<0.05$

Table IV

Estradiol indices in consecutive body fat quartiles of women with positive and negative energy balance. Mean and standard deviation (in parentheses) for four consecutive body fat groups compared by one-way ANOVA tests.

E2 [pmol/l]	All Women N=120	Positive Energy Balance N=70	Negative Energy Balance N=50
mean E2	18.3 (8.92)	17.3 (9.16)	19.6 (10.86)
Very low fat	16.8 (8.58)	14.0 (7.52)*	20.8 (9.06)
Low fat	20.7 (9.61)	20.2 (10.86)	21.3 (7.81)
Average fat	19.7 (8.96)	20.7 (9.80)	18.0 (7.52)
High fat	15.7 (7.86)	13.4 (4.43)*	18.2 (9.96)
mid-cycle E2	21.9 (11.27)	20.2 (11.86)	24.4 (10.12)
Very low fat	19.5 (10.83)*	16.0 (10.32)*	24.3 (10.34)
Low fat	26.1 (11.45)	24.4 (12.48)	28.7 (9.64)
Average fat	24.2 (12.12)	24.7 (13.3)	23.4 (10.45)
High fat	17.7 (8.74)*	14.4 (6.37)*	21.2 (9.76)
day -1 E2	33.1 (17.85)	30.9 (19.1)	35.7 (15.71)
Very low fat	29.4 (15.78)*	24.9 (16.00)*	34.4 (13.49)
Low fat	39.8 (17.67)	39.1 (19.02)	40.77 (16.21)
Average fat	37.1 (21.41)	36.0 (21.87)	38.8 (21.65)
High fat	25.8 (12.93)*	22.1 (14.59)*	29.7 (9.93)
day 0 E2	16.8 (11.21)	14.7 (11.10)	19.9 (10.86)
Very low fat	14.6 (10.24)	11.4(8.10)*	18.7 (12.03)
Low fat	20.0 (11.52)	15.8 (11.25)	26.5 (8.89)
Average fat	19.6 (12.90)	20.8 (14.72)	17.7 (9.81)

High fat	13.1 (8.61)*	9.9 (4.48)*	16.7 (10.80)
mean follicular E2	18.6 (8.94)	17.7 (9.46)	19.7 (8.24)
Very low fat	17.2 (8.33)	14.6 (7.47)*	21.1 (8.68)
Low fat	21.1 (10.30)	21.1 (11.63)	21.1 (8.44)
Average fat	20.3 (9.33)	20.9 (9.93)	19.4 (8.78)
High fat	15.6 (6.53)	13.8 (4.96)*	17.4 (7.62)
mean luteal E2	17.9 (9.67)	16.7 (9.54)	19.5 (9.79)
Very low fat	16.4 (9.16)	13.4 (7.91)*	20.6 (9.78)
Low fat	20.2 (9.79)	19.2 (11.13)	21.6 (7.59)
Average fat	19.0 (9.17)	20.4 (10.36)	16.7 (6.64)
High fat	15.8 (10.34)	13.0 (5.32)*	18.9 (13.44)

* differences between body fat quartiles significant at $p<0.05$

Table V

The association between estradiol indices and body fat percentage in women with positive and negative energy balance and body fat below 31%. Results of simple regression analysis.

	Positive Energy Balance			Negative Energy Balance		
	N	R^2	p	N	R^2	p
Mean E2	55	0.13	0.006	36	0.001	NS
Follicular E2	53	0.11	0.017	36	0.003	NS
Mid-cycle E2	55	0.15	0.003	36	0.010	NS
Day -1 E2	53	0.14	0.006	36	0.039	NS
Day 0 E2	53	0.14	0.005	35	0.010	NS
Luteal E2	55	0.14	0.005	36	0.002	NS

Figure 1

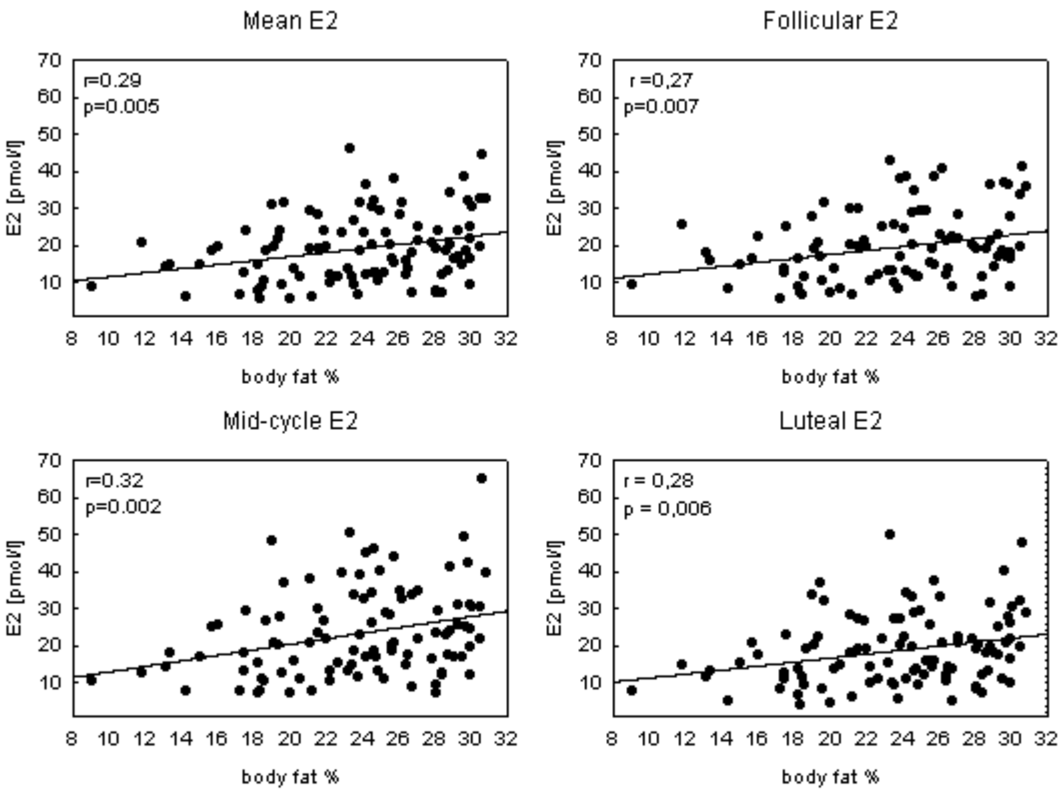

Figure 1. The association between body fat percentage and E2 levels in women with the body fat percentage below 31%.

Figure 2

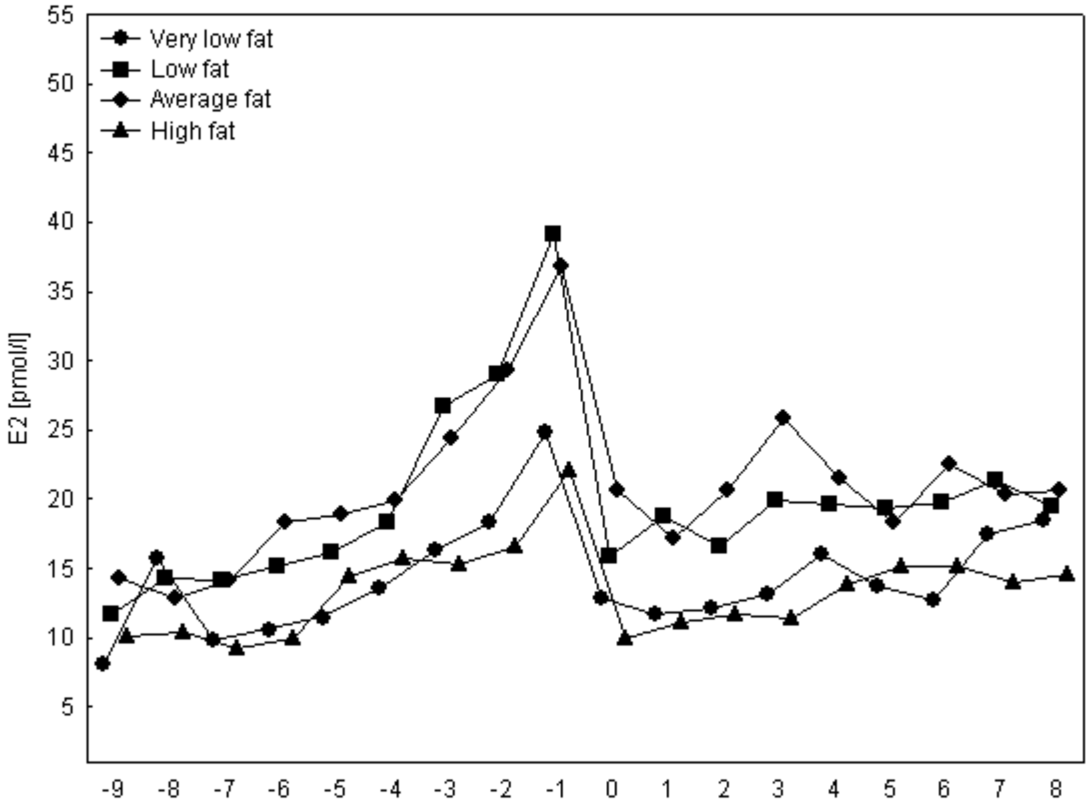

Figure 2. E2 profiles in very low, low, average and high body fat groups of women with positive energy balance.

Figure 3

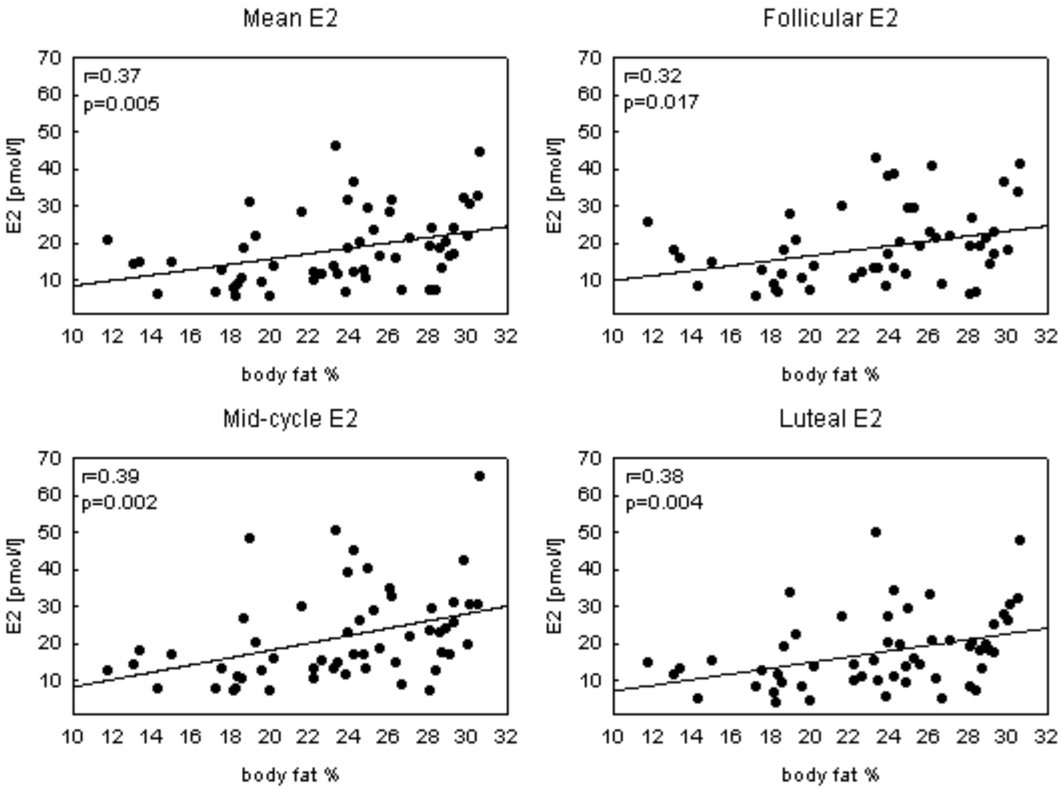

Figure 3. The association between body fat percentage and E2 levels in women with positive energy balance and body fat percentage below 31%.